Alteration in the Activity of Antioxidant Enzymes in *Nigella sativa* Seed during Different Phases of Germination

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Abstract. *Nigella sativa (black cumin; Kalonji is an annual herbaceous plant growing in Western Asia and the Mediterranean region for its seeds which is used as an important spice and condiment. Seed germination is a complex process that involves the activation of specific enzymes at the appropriate times and regulation of their activity. It has been shown that seed germination percentage might be related to the efficiency of free radical scavenging in dry seeds because this scavenging can affect merely seed storage and vigor. Some authors have shown that production of ROS during seed germination may be a beneficial biological reaction, one that is linked with germination capacity, seedling development, and protection against parasitic organisms during germination. For these reason there is a growing interest in the functional role of ROS and corresponding scavenging enzymic systems in seed germination. Antioxidative enzymes such as superoxide dismutase (SOD), POD, and catalase (CAT) are considered to be the main protective enzymes engaged in the removal of free radicals and activated oxygen species. The present study was undertaken to study the morphological changes during germination phases and to assay antioxidant enzymes (SOD and CAT) in each germination stage.*

The seeds were procured from the local market. Seed lots used for the different experiments showed germination capacities ranging from 80 to 98%. For germination studies, seeds were placed on four layers of damp filter paper at 25°C and incubated in dark till the initiation of sprouting after which they were placed at a light intensity of 100 µmol m^{-2} s^{-1} and a 14/10 h (day/night) photoperiod till the complete plantlet with two leaves were obtained. Germination, defined as 1 mm radicle emergence, was followed for 11d; no contamination by microorganisms was observed during this time.

The level of antioxidant enzymes, SOD and CAT decreased gradually in the samples up to 4th day after the start of sprouting there is a rise in enzyme activity as germination proceeds. The activity of SOD and CAT was significantly higher in seed sample as compared to first four days after the start of imbibition. The activity of these two enzymes was seen to increase from fifth day to tenth day. It can be concluded that SOD activity is not correlated with the changes during seed germination. However, its presence in all samples suggests that this enzyme may participate in protection against free superoxide radicals.

Key words: Germination, Free radicals, Antioxidant, SOD, Catalase

1. Introduction

The seeds of *Nigella sativa* have been reported to possess a number of medicinal properties [1, 2]. Seed germination is characterized by imbibition, after which seeds rapidly increase oxygen uptake and oxidative phosphorylation, processes required to meet the high energy cost of germination [3].

Also it has been shown that seed germination percentage might be related to the efficiency of free radical scavenging in dry seeds because this scavenging can affect merely seed storage and vigor [4,5]). Some authors have shown that production of ROS during seed germination may be a beneficial biological reaction, one that is linked with germination capacity, seedling development, and protection against parasitic organisms during germination [6]. For these reason there is a growing interest in the functional role of ROS and corresponding scavenging enzymic systems in seed germination [7, 8]. Antioxidative enzymes such as superoxide dismutase (SOD), POD, and catalase (CAT) are considered to be the main protective enzymes

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engaged in the removal of free radicals and activated oxygen species [9]. Catalase and SOD are the most efficient antioxidative enzymes [10). On the other hand, PODs also have a role in very important physiological processes like control of growth by lignification, cross-linking of pectins and structural proteins in the cell wall, and catabolism of auxins [11]. Despite the importance of PODs in plant development, their exact relationship to developmental events is often obscured by their extensive polymorphism in a single plant species. It is therefore very important to select POD associated with plant development for purification and further studies [12]. Studies of antioxidative enzymes during germination of coniferous trees are rather rare. One such study treated enzymes involved in cycling of ascorbic acid and glutathione in *Pinus pinea* seeds during the first stages of germination [3]. This work is the first study of the activities and isoenzyme pattern of the antioxidative enzymes CAT, POD, and SOD during germination of *P. omorika* seeds. Our aim was to follow the expression of particular parts of antioxidative systems during the early stages of germination of two genetically different lines and compare them with the activities in the needles of Serbian spruce. We also sought to find out if there is a correlation between activities of these antioxidative enzymes and seed germination in these two lines.

2. Materials and methods

Collection of N*igella sativa* seeds

Seeds of *N. sativa* were procured in December, 2009 from a local grocery store in Lucknow, India and surface sterilized with 1% $HgCl_2$ for 30 minutes.

Germination of seeds

The seeds were procured from the local market. Seed lots used for the different experiments showed germination capacities ranging from 80 to 98%. For germination studies, seeds were placed on four layers of damp filter paper at 25°C and incubated in dark till the initiation of sprouting after which they were placed at a light intensity of 100 µmol m^{-2} $s^{-1}$and a 14/10 h (day/night) photoperiod till the complete plantlet with two leaves were obtained. Germination, defined as 1 mm radicle emergence, was followed for 11d; no contamination by microorganisms was observed during this time.

Antioxidant enzyme assays

Biochemical evaluations were performed to determine the activity of superoxide dismutase (SOD) and catalase (CAT).

Preparation of enzyme extracts

Different enzymes were assayed in each germinating stage of the seed. For preparation of crude extract, 0.25 gram of plant material was homogenized in chilled mortar and pestle with ice cold 5 ml of 50mM phosphate buffer (pH-7.8). Homogenates were centrifuged for 10 min at 10,000 rpm at 4°C and supernatant was collected which was used for the assays. Enzyme activities were referred in terms of fresh weight.

SOD activity was determined by measurement of inhibition of photochemical reduction of nitro blue tetrazolium (NBT) at 560 nm [13]. The 3 mL reaction mixture contained 50 mmol/L phosphate buffer (pH=7.8), 0.1 mmolg/L ethylenediaminetetra-acetic-acid (EDTA), 13 mmol/L methionine, 75 umol/L NBT, 16.7 umol/L riboflavin and enzyme extract. Riboflavin was added at last and the reaction was initiated by placing the tubes under two 9 W fluorescent lamps. The reaction was determined after 15 min by removal from the light source. An illuminated blank without protein gave the maximum reduction of NBT, therefore, the maximum absorbance at 560 nm. SOD activity is present as absorbance of sample divided by absorbance for blank, giving the percentage of inhibition. One unit of SOD activity was defined as the amount of enzyme required for the inhibition of the photochemical reduction of NBT by 50%.

Catalase activity was assayed in a reaction solution (3 ml) containing 50 mM phosphate buffer (pH 7.0), 30% (w/v) H_2O_2 and 0.5 ml of enzyme extract [14]. The reaction was started by the addition of enzyme extract. The activity of catalase was estimated by the decrease of absorbance at 240 nm for 1 minute as a consequence of H_2O_2 consumption. The extinction coefficient for H_2O_2 was 4.32 cm^2/µmol.

Statistical Analysis

Every experiment was repeated thrice and all the results were expressed as mean value ±SD for three replications. For each replication plant material was taken by weight from different stages of germination.

2. Results and discussions

The decrease in catalase enzyme activity was detected in *N. sativa* seeds up to 4th day after the start of sprouting there is a rise in enzyme activity as germination proceeds. Activity of SOD did not change significantly during germination, It can be concluded that SOD activity is not correlated with the changes during seed germination. However, its presence in all samples suggests that this enzyme may participate in protection against free superoxide radicals. Under Cadmium stress, the pattern of antioxidant enzyme activity was similar to that of normal germination but the level of enzymes was reduced during earlier stages of germination which increased from 9th day till the formation of plantlet.

Table 1. Activities of antioxidant enzymes in terms of unit per mg of fresh weight.

DAY	SOD	CAT
0	453.0±0.81	3715.10±0.70
1	446.2±0.48	3302.45±0.49
2	423.3±0.25	2064.65±0.63
3	365.5±0.32	1650.80±0.84
4	351.4±0.59	2890.30±0.42
5	359.6±0.42	3715.75±0.21
6	395.7±0.27	4541.55±0.49
7	407.2±0.65	5779.75±0.35
8	416.6±0.63	6605.30±0.42
9	407.5±0.79	7431.55±0.49
10	497.4±0.70	8669.40±0.56
11	474±0.28	8256.70±0.14

The level of antioxidant enzymes, SOD and CAT decreased gradually in the samples from first day to fourth day. The activity of SOD and CAT was significantly higher in seed sample as compared to first four days after the start of imbibition. The activity of these two enzymes was seen to increase from fifth day to tenth day. The complete seedling was formed on eleventh day of germination and the activities of SOD and CAT was lesser in seedling as compared to tenth day of germination (Table 1). This result is in compliance with the research of scientists and published data. The level of the antioxidants, SOD and CAT decreased gradually in all the seeds during the first five days of germination. The activity of SOD and CAT in fenugreek was significantly higher in the seeds treated with 0.05% carbendazim than in the control seeds ($p>0.01$). The decrease in the levels of SOD and CAT in the presence of 0.1% and 0.3% carbendazim could be attributed to the increased utilization of these antioxidants to combat the reactive oxygen species produced excessively during the oxidative stress [15].

Another research which supports the above result is shown by another group of researchers in *P. omorika seeds*. As no changes in enzyme activity were detected in *P. omorika* seeds up to 4th day after the start of imbibition, we here present specific activity of the enzymes catalase, superoxide dismutase, and peroxidase from the 4th day, when most of the seeds germinated [16].

4. References

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